

REMARKS

The Examiner has restricted this application to one of the following inventions under 35 U.S.C. § 121:

- I. Claims 1-15, drawn to a method of identifying and selecting transformants comprising transforming a host cell with Agrobacterium carrying a vector containing a targeting construct, whereby recombination occurs, and selecting transformants, classified in class 435, subclass 471.
- II. Claims 16-20, 64 and 65, drawn to a strain of fungal cells transformed with a vector containing a targeting construct, and the vector, and Agrobacterium comprising the vector, classified in class 435, subclass 254.11, 252.2, 320.1.
- III. Claims 21-35, drawn to a method of identifying a gene knock out mutant comprising providing a polynucleotide construct, introducing into Agrobacterium the construct, incubating Agrobacterium produced with fungal cells under conditions so that the construct is integrated into a fungal cell genome wherein transformant resulting from knockout lack a negative selection marker, and ectopic heterologous or illegitimate transformants express both a negative and positive selection marker, selecting knockout mutants by subjecting transformed cells to a positive and a negative selection action, classified in class 435, subclass 471.
- III. Claim 36, drawn to a strain of transformed fungal cells comprising knockout mutations, classified in class 435, subclass 254.11.
- IV. Claims 37-52, drawn to a method of transforming fungal cells to identify mutants comprising inserting a polynucleotide construct into an Agrobacterium-based vector between T-DNA borders, introducing said vector into Agrobacterium tumefaciens,

wherein said cells contain a virulence region, inducing [introducing?] virulence genes to T-DNA containing said construct from said *Agrobacterium tumefaciens* and incubating said *Agrobacterium tumefaciens* with fungal cells to be transformed and selecting transformed cells from untransformed cells by subjecting transformants to a positive and a negative selection agent, classified in class 435, subclass 471.

- V. Claim 53, drawn to a strain of transformed fungal cells comprising T-DNA with virulence genes, classified in class 435, subclass 254.11.
- VI. Claims 54-58, drawn to a method of identifying and selecting transformants comprising transforming fungal cells with *A. tumefaciens* under conditions whereby recombination occurs, wherein transformants resulting from a gene knockout lack a negative selection marker and ectopic, heterologous or illegitimate transformants will express a negative and a positive selection marker, said *A. tumefaciens* comprising a gene disruption vector, said vector comprises a polynucleotide encoding a negative selection marker linked to a fragment of DNA flanked by DNA sequence homologous to the polynucleotide to be targeted, wherein said fragment contains at least one mutant allele, wherein said mutant allele is generated by the insertion of a positive selection marker; regenerating transformants in the presence of both a positive and a negative selection agent, and selecting putative knockout mutants, classified in class 435, subclass 471.
- VI. Claims 59, drawn to a strain of transformed fungal cells with knockout mutations, classified in class 435, subclass 254.11.
- VII. Claims 60-61, drawn to a method of identifying and selecting transformants comprising transforming fungal cells with *A. tumefaciens* cells under conditions whereby recombination occurs wherein transformants resulting from gene knockout lack a

negative selection marker, said vector comprising a pGreen II cloning site, a polynucleotide sequence that encodes a negative selection marker, said sequence is linked to a fragment of DNA, wherein said DNA fragment is disrupted by a positive selection marker; and selecting gene knockout mutants by subjecting transformed fungal cells to a positive and a negative selection agent, classified in class 435, subclass 471.

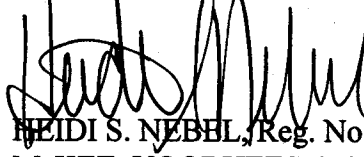
- VII. Claim 62, drawn to a polynucleotide having a DNA fragment disrupted by a positive selectable marker in a targeted polynucleotide, classified in class 536, subclass 23.1.
- VIII. Claim 63, drawn to a polynucleotide comprising a first polynucleotide sequence encoding a negative selection marker; a DNA fragment disrupted by a positive selection marker, and a pGreen II cloning site, classified in class 536, subclass 23.1.

Applicants provisionally elect, with traverse, Group I, Claims 1-15, drawn to a method of identifying and selecting transformants comprising transforming a host cell with *Agrobacterium* carrying a vector containing a targeting construct, whereby recombination occurs, and selecting transformants, classified in class 435, subclass 471.

No fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

A handwritten signature in black ink, appearing to read 'Heidi S. Nebel', written over the printed name.

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